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Optical Imaging of Oesophageal cancer

Optisk avbildning av øsofagkreft og Barretts øsofag

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Optical imaging of oesophageal cancer and Barrett's oesophagus**Field of the invention**

5 The present invention provides contrast agents for optical imaging of oesophageal cancer and Barrett's oesophagus in patients. The contrast agents may be used in diagnosis of oesophageal cancer and Barrett's oesophagus, for follow up of progress in disease development, and for follow up of treatment of oesophageal cancer and Barrett's oesophagus.

10 The present invention also provides new methods of optical imaging of oesophageal cancer and Barrett's oesophagus in patients, for diagnosis and for follow up of disease development and treatment of oesophageal cancer and Barrett's oesophagus.

15 Description of related art

Oesophageal cancer is not among the most frequent forms of cancer and less than 5% of all reported cancer cases are oesophageal cancer. However, 30 000 new cases are diagnosed per year in USA. Oesophageal cancer is predominantly a disease of the male. The occurrence of the disease varies from country to country 20 with high occurrence in for example India, Japan, Russia, China, UK and Middle East.

The main risk factors for oesophageal cancer include tobacco, alcohol and the diet. 25 Oesophageal cancer is divided into two major types, squamous cell carcinoma and adenocarcinoma, depending on the type of cells that are malignant. Barrett's oesophagus is a premalignant condition and the presence is associated with increased risk for development of oesophageal cancer; especially adenocarcinoma. Chronic reflux increases risk for Barrett's oesophagus, it has therefore been suggested that gastro oesophageal reflux (GERD) is a risk factor for oesophageal 30 cancer.

Adenocarcinoma of the oesophagus is more prevalent than squamous cell carcinoma in US and Western Europe.

35 Oesophageal cancer can be a treatable disease but is rarely curable. The overall 5-year survival rate is between 5% and 30%. Data from US show a 5-year survival rate of about 5%. Early diagnosis of oesophageal cancer improves the survival rate of the

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patient. Primary treatment includes surgery alone or chemotherapy in combination with radiation. Chemotherapy used in treatment of oesophageal cancer includes 5-fluorouracil and cisplatin. Lack of precise pre-operative staging is a major clinical problem.

5

US 6,035,229 (Washington Research Foundation) describes a system for detecting Barrett's oesophagus utilizing an illumination and imaging probe at the end of a catheter. The document does not disclose any optical contrast agent.

10 US 5,888,743 (Das) describes an in vitro method for the diagnosis of benign Barrett's epithelium and Barrett's derived adenocarcinoma comprising a monoclonal antibody that reacts with cells.

15 US 4,243,652 (The Procter & Gamble Company) describes a gastrointestinal scanning agent also to be used for visualization of the oesophageal entry. The agent comprises a gamma radiation emitting radionuclide.

20 Oesophageal cancer and Barrett's oesophagus are still a challenge to diagnose and treat. There is still need for improved diagnostic methods, especially for diagnosis of oesophageal cancer and Barrett's oesophagus in an early stage with good reliability. Surprisingly, we have discovered that the use of optical imaging methods and new contrast agents fulfil these requirements.

Summary of the invention

25 In view of the needs of the art the present invention provides an optical imaging contrast agent with affinity for an abnormally expressed biological target associated with oesophageal cancer and Barrett's oesophagus.

The invention is also described in the claims.

30

The following definitions will be used throughout the document:

35 Oesophageal cancerous tissue: The condition includes alterations in the oesophageal tissue. This includes oesophageal tissue that shows metaplastic alterations characteristic for Barrett's oesophagus, such as areas of columnar instead of squamous epithelium. Metaplastic oesophageal tissue in general, particularly tissue that shows progression towards malignancy, involving larger parts of the

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oesophagus and including invasion of adjacent tissue are also included. Metastases from oesophageal carcinoma are also considered as oesophageal cancerous tissue.

5 Abnormally expressed target: A target that is either overexpressed or downregulated in diseased tissue.

Overexpressed target: A receptor, an enzyme or another molecule or chemical entity that is present in a higher amount in diseased tissue than in normal tissue.

10 Downregulated target: A receptor, an enzyme or another molecule or chemical entity that is present in a lower amount in diseased tissue than in normal tissue.

Detailed description of the invention

A first aspect of the present invention is an optical imaging contrast agent for imaging 15 of oesophageal cancer and Barrett's oesophagus. By the term optical imaging contrast agent, or just contrast agent, we mean a molecular moiety used for enhancement of image contrast *in vivo* comprising at least one moiety that interacts with light in the ultraviolet, visible or near-infrared part of the electromagnetic spectrum.

20 The contrast agent has affinity for an abnormally expressed target associated with oesophageal cancer or Barrett's oesophagus. That is, the contrast agent has affinity for a target that is either downregulated or overexpressed in oesophageal cancerous tissue.

25 Oesophageal cancerous tissue containing a downregulated target is identified by a low amount of bound imaging agent compared to normal tissue. In this situation, the amount of imaging agent should be less than 50 % of that in normal tissue, preferably less than 10 %.

30 Preferred contrast agents according to the invention, have affinity for an overexpressed target associated with oesophageal cancer or Barrett's oesophagus. Preferred targets are those targets that are more than 50 % more abundant in oesophageal cancerous tissue than in surrounding tissue. More preferred targets are 35 those targets that are more than two times more abundant in oesophageal cancerous tissue than in surrounding tissue. The most preferred targets are those targets that

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are more than 5 times more abundant in oesophageal cancerous tissue than in surrounding tissue.

In a further aspect of the invention, targets that are mutated in oesophageal
5 cancerous tissue can be identified by lack of binding of an imaging agent that does bind to normal tissue; alternatively, the imaging agent might be directed specifically towards the mutated target, and binding to normal tissue would be minimal. The mutated target can be a protein in oesophageal cancerous tissue that is altered as a result of a germline or somatic mutation, and including alterations resulting from
10 differential splicing of RNA and changes in post-translational modifications, particularly glycosylation patterns, but not limited to these types of alterations.

Relevant groups of targets are receptors, enzymes, nucleic acids, proteins, lipids, other macromolecules like for example lipoproteins and glycoproteins. The targets
15 may be located in the vascular system, in the extracellular space, associated with cell membranes or located intracellularly.

Preferred groups of targets are antigens, proteins involved in cell cycle regulation or intracellular signalling, enzymes, hormones, growth factors, cytokines and similar
20 proteins and peptides, cytokeratins, cell-surface receptors associated with Barrett's oesophagus or oesophageal cancer.

The following biological targets are preferred targets for contrast agents for optical imaging of oesophageal cancer and Barrett's oesophagus:

25 Targets that are overexpressed in Barrett's oesophagus:

Antigens:

MUC5AC, MUC3, MUC2, MUC6, MUC2, CD34, PCNA, MUC2, Sulfo-Lewis(a).

30 Proteins involved in cell cycle regulation or intracellular signalling:

PCNA, enzymes of polyamine metabolism, p53, p63, KI67, p53, c-ras, c-src, β-catenin, Mcm2, Mcm5.

Hormones, growth factors, cytokines and similar proteins and peptides:

35 VEGFs, IL1β, IL-8, IL-10, TGF-α, EGF, TGF-α, TNF-α.

Cytokeratins etc.:

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CK7, CK20, CK 8, CK 13, CK 18, CK 19.

Cell-surface receptors:

Epidermal growth factor receptor (EGFR), c-erb2, CD44H, CD44V6, c-myc, Guanylyl
5 cyclase.

Others:

c-jun, E-cadherin, β-galactosidase, metallothionein, telomerase.

10 More preferred targets that are overexpressed in Barrett's oesophagus are E-cadherin, Guanylyl cyclase and epidermal growth factor receptor (EGFR).

Targets that are downregulated in Barrett's oesophagus:

MUC1, Glutathione S-transferase, Retinoblastoma gene product.

15 A more preferred target that is downregulated in Barrett's oesophagus is MUC1.

Targets that are overexpressed in squamous cell carcinoma of the oesophagus:

20 Antigens and cell-surface receptors:

CD44, CD44v2, CD44v6, Squamous cell carcinoma antigen (SCC), P62/c-myc (HGF receptor), c-erb2 (EGF receptor).

Proteins involved in cell cycle regulation or intracellular signalling:

25 MIB-1, P53, PCNA, survivin, CDC25A, CDC25B, cyclin D1, MDM2, p21..

Cytokeratins etc.:

CK 5/6, CK8, CK10, CK 13, CK18, CK19.

30 Others:

Endothelin, bFGF, proteins involved in Angiogenesis, involucrin, Cathepsin D.

More preferred targets that are overexpressed in squamous cell carcinoma of the oesophagus are: CD44, squamous cell carcinoma antigen, P62/c-myc (HGF receptor) and c-erb2 (EGF receptor).

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Targets that are downregulated in squamous cell carcinoma of the oesophagus:

Nm23-H1, E-cadherin, pRb, cyclin D1, axin, RCAS1, CK 20 and PAX9 gene product.

5 More preferred targets that are downregulated in squamous cell carcinoma of the oesophagus are E-cadherin and CK20.

Targets that are overexpressed in Barrett's adenocarcinoma and other carcinomas of the oesophagus:

10 Antigens and cell-surface receptors:

β-catenin, CD44V6, SCC, Tumor M2-PK, c-erb2, c-myc, EGFR, Guanylyl cyclase, ligands of Helix pomatia lectin, MUC1, MUC4, Epidermal growth factor receptor, c-erb2.

15 Enzymes:

Cox-2, MMP-1, MMP-2, MMP-7, MMP-9, MMP-12, Cathepsin D, Pyrimidine nucleoside phosphorylase.

Others:

20 TNF-α, CK7, involucrin, EF1 gamma, Mcm2, Mcm5, Ki-67, p53, TGF-α, EGF, FGF-1, c-src, c-ras.

More preferred targets that are overexpressed in Barrett's adenocarcinoma and other carcinomas of the oesophagus are matrix metalloproteinases, Cox-2 and EGFR.

25

Targets that are downregulated in Barrett's adenocarcinoma and other carcinomas of the oesophagus:

CK20, E-cadherin, Lamins A/C and B1, nm23.

30 More preferred targets that are downregulated in Barrett's adenocarcinoma and other carcinomas of the oesophagus are E-cadherin and CK20.

More preferred targets for both oesophageal cancer and Barrett's oesophagus are:

E-cadherin, CD44, Squamous cell carcinoma antigen, D, COX-2, matrix

35 metalloproteinases, bombesin receptor, endothelin receptor, P62/c-myc (HGF receptor), p53 and EGFR/erB-2.

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Generally, any targets that have been identified as possible targets for agents for treatment of oesophageal cancer and Barrett's oesophagus are potential targets also in optical imaging.

5 The preferred contrast agents of the present invention are molecules with relatively low molecular weights. The molecular weight of more preferred contrast agents is below 10000 Daltons, more preferably below 7000 Daltons.

10 The contrast agents are preferably comprised of a vector that has affinity for an abnormally expressed target in oesophageal cancerous tissue, and an optical reporter.

Thus viewed from one aspect the present invention provides a contrast agent of formula I:

15 V-L-R (I)

wherein V is one or more vector moieties having affinity for one or more abnormally expressed target in oesophageal cancerous tissue, L is a linker moiety or a bond and R is one or more reporter moieties detectable in optical imaging.

20 The vector has the ability to direct the contrast agent to a region of oesophageal cancer and Barrett's oesophagus. The vector has affinity for the abnormally expressed target and preferably binds to the target. The reporter must be detectable in an optical imaging procedure and the linker must couple vector to reporter, at least until the reporter has been delivered to the region of oesophageal cancer and

25 Barrett's oesophagus and preferably until the imaging procedure has been completed.

30 The vector can generally be any type of molecule that has affinity for abnormally expressed target. The molecules should be physiologically acceptable and should preferably have a certain degree of stability. The vector can be selected from the following group of compounds: peptides, peptoids/peptidomimetics, oligonucleotides, oligosaccharides, fat-related compounds, like fatty-acids, traditional organic drug-like small molecules, synthetic or semi-synthetic, and derivatives and mimetics thereof.

35 When the target is an enzyme the vector may comprise an inhibitor of the enzyme. The vector of the contrast agent should preferably have a molecular weight of less than 4500 Daltons and more preferably less than 2500 Daltons.

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Contrast agents having affinity for more than one abnormally expressed target related to the disease is an aspect of the invention. Such contrast agents can comprise two or more different vectors or molecular subunits that target two or more different abnormally expressed targets.

Another possibility according to the present invention is that the contrast agent comprises one vector that is able to bind to more than one abnormally expressed target in oesophageal cancer and Barrett's oesophagus.

10

A contrast agent according to the present invention can also comprise more than one vector of same chemical composition that bind to the abnormally expressed biological target.

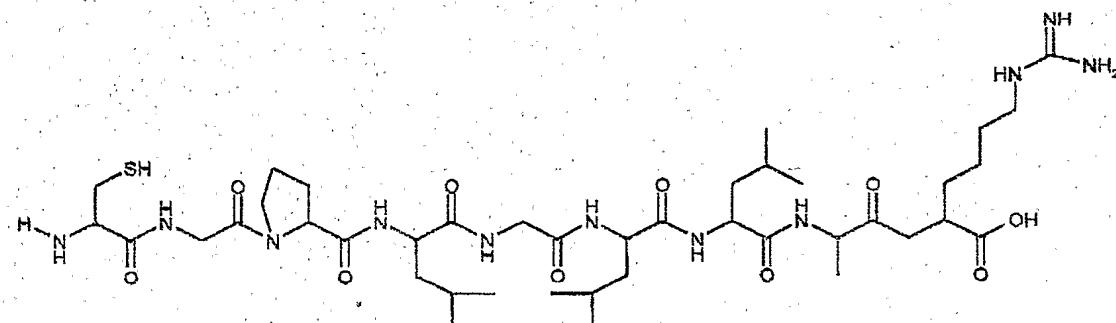
15

Below are some examples of vectors having affinity for oesophageal cancerous tissue-related abnormally expressed targets:

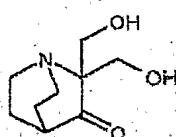
Vectors for matrix metalloproteinases, especially for MMP-7:

Peptide sequence: Cys-Gly-Pro-Leu-Gly-Leu-Leu-Ala-Arg-OH

20



Vectors for p53:

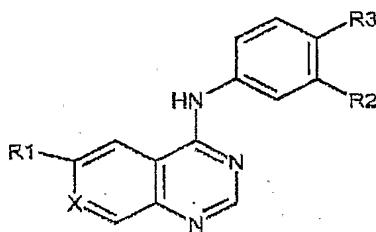


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Vectors for EGFR/erB-2:



5 Wherein

R1 = substituted alkoxy, arylamide,

R2 = halogen,

R3 = H, fluorine,

X = N or CR4, wherein R4 is alkoxy.

10

Vectors for COX-2:

Arachidonic acid:



15

Arachidonic acid is the endogenous substrate for COX-2.

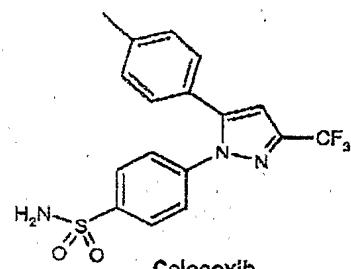
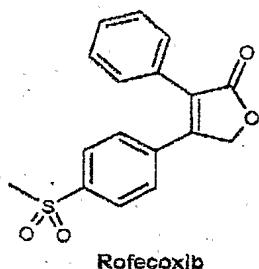
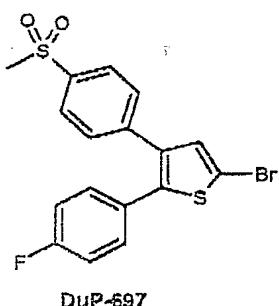
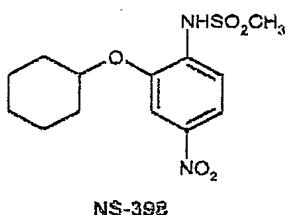
20

Other vectors for COX-2 are exogenous compounds that bind to COX-2, for example so-called COX-2 inhibitors. The chemical classes of the main COX-2 inhibitors are shown in WO 02/07721.

Such vectors include:

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A wide variety of linkers can be used. The linker component of the contrast agent is at its simplest a bond between the vector and the reporter moieties. In this aspect the reporter part of the molecule is directly bound to the vector that binds to the abnormally expressed target. More generally however the linker will provide a mono-

5 or multi-molecular skeleton covalently or non-covalently linking one or more vectors to one or more reporters, e.g. a linear, cyclic, branched or reticulate molecular skeleton, or a molecular aggregate, with in-built or pendant groups which bind covalently or non-covalently, e.g. coordinatively, with the vector and reporter moieties. The linker group can be relatively large in order to build into the contrast agent optimal size or optimal shape or simply to improve the binding characteristics for the contrast agent to the abnormally expressed target in oesophageal cancer and Barrett's oesophageal tissue.

15

Thus linking of a reporter unit to a desired vector may be achieved by covalent or non-covalent means, usually involving interaction with one or more functional groups located on the reporter and/or vector. Examples of chemically reactive functional groups which may be employed for this purpose include amino, hydroxyl,

20 sulphhydroxyl, carboxyl and carbonyl groups, as well as carbohydrate groups, vicinal diols, thioethers, 2-aminoalcohols, 2-aminothiols, guanidinyl, imidazolyl and phenolic groups.

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The reporter moieties in the contrast agents of the invention may be any moiety capable of detection either directly or indirectly in an optical imaging procedure. The reporter might be a light scatterer (e.g. a coloured or uncoloured particle), a light absorber or a light emitter. More preferably the reporter is a dye such as a chromophore or a fluorescent compound. The dye part of the contrast agent can be any dye that interacts with light in the electromagnetic spectrum with wavelengths from the ultraviolet light to the near-infrared. Preferably the contrast agent of the invention has fluorescent properties.

5 Preferred organic dye reporters include groups having an extensive delocalized electron system, e.g. cyanines, merocyanines, indocyanines, phthalocyanines, naphthalocyanines, triphenylmethines, porphyrins, pyrilium dyes, thiapyrilium dyes, squarylium dyes, croconium dyes, azulenium dyes, indoanilines, benzophenoxyazinium dyes, benzothiaphenothiazinium dyes, anthraquinones, 10 napthoquinones, indathrenes, phthaloylacridores, trisphenoquinones, azo dyes, intramolecular and intermolecular charge-transfer dyes and dye complexes, tropones, tetrazines, bis(dithiolene) complexes, bis(benzene-dithiolate) complexes, iodoaniline dyes, bis(S,O-dithiolene) complexes. Fluorescent proteins, such as green 15 fluorescent protein (GFP) and modifications of GFP that have different absorption/emission properties are also useful. Complexes of certain rare earth metals (e.g., europium, samarium, terbium or dysprosium) are used in certain contexts, as are fluorescent nanocrystals (quantum dots).

20 Particular examples of chromophores which may be used include fluorescein, sulforhodamine 101 (Texas Red), rhodamine B, rhodamine 6G, rhodamine 19, indocyanine green, Cy2, Cy3, Cy3.5, Cy5, Cy5.5, Cy7, Marina Blue, Pacific Blue, Oregon Green 488, Oregon Green 514, tetramethylrhodamine, and Alexa Fluor 350, Alexa Fluor 430, Alexa Fluor 532, Alexa Fluor 546, Alexa Fluor 555, Alexa Fluor 568, Alexa Fluor 594, Alexa Fluor 633, Alexa Fluor 647, Alexa Fluor 660, Alexa Fluor 680, 25 Alexa Fluor 700, and Alexa Fluor 750.

30 Particularly preferred are dyes which have absorption maxima in the visible or near-infrared region, between 400 nm and 3 µm, particularly between 600 and 1300 nm.

35 The contrast agents according the invention can comprise more than one dye molecular sub-unit. These dye sub-units might be similar or different from a chemical point of view. Preferred contrast agents have less than 6 dye molecular sub-units.

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Several relevant targets for oesophageal cancer and Barrett's oesophagus are enzymes. A contrast agent for optical imaging of oesophageal cancer and Barrett's oesophagus for targeting an enzyme can be an enzyme contrast agent substrate that

5 can be transformed to a contrast agent product possessing different pharmacokinetic and/or pharmacodynamic properties from the contrast agent substrate. This embodiment of the invention provides contrast agent substrates having affinity for an abnormally expressed enzyme, wherein the contrast agent substrate changes pharmacodynamic and/or pharmacokinetic properties upon a chemical modification

10 into a contrast agent product in a specific enzymatic transformation, and thereby enabling detection of areas of disease upon a deviation in the enzyme activity from the normal. Typical differences in pharmacodynamic and/or pharmacokinetic properties can be binding properties to specific tissue, membrane penetration properties, protein binding and solubility issues.

15

Alternatively, if the abnormally expressed target for diagnosis of oesophageal cancer and Barrett's oesophagus is an enzyme, the contrast agent for optical imaging can be a dye molecule that directly binds to the enzyme. The contrast agent will have affinity for the abnormally expressed enzyme, and this may be used to identify tissue

20

or cells with increased enzymatic activity.

25

In a further aspect of the invention the contrast agent changes dye characteristics as a result of an enzymatic transformation. For example, a fluorescent dye reporter of the contrast agent is quenched (no fluorescence) by associated quencher groups, until an enzymatic cleavage takes place, separating the dye from the quencher groups and resulting in fluorescence at the site of the abnormally expressed enzyme.

30

Another aspect of this part of the invention is that the dye may change colour, as e.g. a change in absorption and/or emission spectrum, as a result of an enzymatic transformation.

35

If the abnormally expressed target for diagnosis of oesophageal cancer and Barrett's oesophagus is a receptor or another non-catalytical target, the contrast agent for optical imaging can bind directly to the target and normally not change the dye characteristics.

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The preferred contrast agents of the present invention are soluble in water. This means that the preferred contrast agents have a solubility in water at pH 7.4 of at least 1 mg/ml.

5 The contrast agents of the present invention can be identified by random screening, for example by testing of affinity for abnormally expressed targets of a library of dye labelled compounds either prepared and tested as single compounds or by preparation and testing of mixture of compounds (a combinatorial approach). Alternatively, random screening may be used to identify suitable vectors, before
10 labelling with a reporter.

The contrast agents of the present invention can also be identified by use of technology within the field of intelligent drug design. One way to perform this is to use computer-based techniques (molecular modelling or other forms of computer-aided drug design) or use of knowledge about natural and exogenous ligands (vectors) for the abnormally expressed targets. The sources for exogenous ligands can for example be the chemical structures of therapeutic molecules for targeting the same target. One typical approach here will be to bind the dye chemical sub-unit to the targeting vector so that the binding properties of the vector are not reduced. This can
20 be performed by linking the dye at the far end away from the pharmacophore centre (the active targeting part of the molecule).

The contrast agents of the invention are preferably not endogenous substances alone. Some endogenous substances, for instance estrogen, have certain
25 fluorescent properties in themselves, but they are not likely to be sufficient for use in optical imaging. Endogenous substances combined with an optical reporter however, fall within the contrast agents of the invention.

The contrast agents of the invention are intended for use in optical imaging. Any method that forms an image for diagnosis of disease, follow up of disease development or for follow up of disease treatment based on interaction with light in the electromagnetic spectrum from ultraviolet to near-infrared radiation fall within the term optical imaging. Optical imaging further includes all methods from direct visualization without use of any device and use of devices such as various scopes, catheters and optical imaging equipment, for example computer based hardware for tomographic presentations. The contrast agents will be useful with optical imaging modalities and measurement techniques including, but not limited to: luminescence
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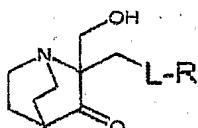
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imaging; endoscopy; fluorescence endoscopy; optical coherence tomography; transmittance imaging; time resolved transmittance imaging; confocal imaging; nonlinear microscopy; photoacoustic imaging; acousto-optical imaging; spectroscopy; reflectance spectroscopy; interferometry; coherence interferometry; diffuse optical

5 tomography and fluorescence mediated diffuse optical tomography (continuous wave, time domain and frequency domain systems), and measurement of light scattering, absorption, polarisation, luminescence, fluorescence lifetime, quantum yield, and quenching.

10 Some examples of contrast agents for optical imaging of oesophageal cancer and Barrett's oesophagus according to the invention are shown below:

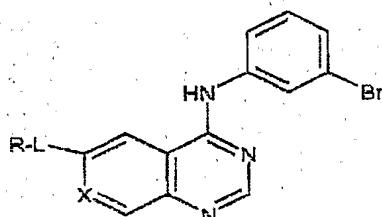
Contrast agent with affinity for p53:



15

wherein L is a linker and R is a reporter according to the invention.

Contrast agent with affinity for EGFR/erB-2:

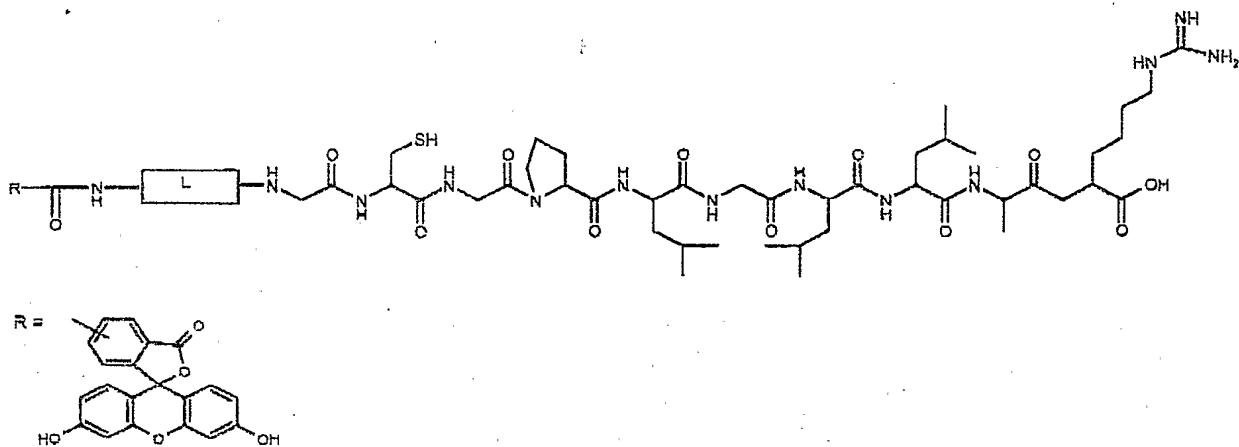


20

wherein X is N or CR4, wherein R4 is alkoxy, and wherein L is a linker and R a reporter according to the invention.

25 Contrast agent for mapping of matrix metalloproteinase wherein the vector peptide is linked to fluorescein through a linker:

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A further embodiment is the use of contrast agents of the invention for optical

5 imaging of oesophageal cancer and Barrett's oesophagus, that is for diagnosis of oesophageal cancer and Barrett's oesophagus, for use in follow up the progress in oesophageal cancer and Barrett's oesophagus development or for follow up the treatment of oesophageal cancer and Barrett's oesophagus.

10 In the context of this invention, diagnosis includes screening of selected populations, early detection, biopsy guidance, characterisation, staging, grading, therapy efficacy monitoring, long-term follow-up of relapse and surgical guidance.

15 Still another embodiment of the invention is a method of optical imaging of oesophageal cancer and Barrett's oesophagus using the contrast agents as described.

20 Still another embodiment of the invention is a method of optical imaging for diagnosis, to follow up the progress of oesophageal cancer and Barrett's oesophagus development and to follow up the treatment of oesophageal cancer and Barrett's oesophagus, using a contrast agent as described.

25 One aspect of these methods is to administer the present contrast agents and follow the accumulation and elimination directly visually during surgery. Another aspect of these methods is to administer the present contrast agents and perform visual diagnosis through a gastroscope.

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Still another aspect of the present invention is to administer the present contrast agents and perform the image diagnosis using computerized equipment as for example a tomograph.

5 Still another embodiment of the invention is use of a contrast agent as described for the manufacture of a diagnostic agent for use in a method of optical imaging of oesophageal cancer and Barrett's oesophagus involving administration of said diagnostic agent to an animate subject and generation of an image of at least part of said body.

10

Still another embodiment of the invention is pharmaceutical compositions comprising one or more contrast agents as described or pharmaceutically acceptable salts thereof for optical imaging for diagnosis of oesophageal cancer and Barrett's oesophagus, for follow up progress of oesophageal cancer and Barrett's oesophagus 15 development or for follow up the treatment of oesophageal cancer and Barrett's oesophagus. The contrast agents of the present invention may be formulated in conventional pharmaceutical or veterinary parenteral administration forms, e.g. suspensions, dispersions, etc., for example in an aqueous vehicle such as water for injections. Such compositions may further contain pharmaceutically acceptable 20 diluents and excipients and formulation aids, for example stabilizers, antioxidants, osmolality adjusting agents, buffers, pH adjusting agents, etc. The most preferred formulation is a sterile solution for intravascular administration or for direct injection into area of interest. Where the agent is formulated in a ready-to-use form for parenteral administration, the carrier medium is preferably isotonic or somewhat 25 hypertonic.

25

The dosage of the contrast agents of the invention will depend upon the clinical indication, choice of contrast agent and method of administration. In general, however dosages will be between 1 micro gram and 70 grams and more preferably between 10 micro grams and 5 grams for an adult human.

30

While the present invention is particularly suitable for methods involving parenteral administration of the contrast agent, e.g. into the vasculature or directly into an organ or muscle tissue, intravenous administration being especially preferred, it is also applicable where administration is not via a parenteral route, e.g. where 35 administration is transdermal, nasal, sub-lingual or is into an externally voiding body

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cavity, e.g. the gastrointestinal tract. The present invention is deemed to extend to cover such administration.

The following examples are illustrative only and not intended to be limiting. Other
5 features and advantages of the invention will be apparent from the detailed
description and from the claims.

Examples:

Example 1. Contrast agent for mapping of matrix metalloproteinase (MMP).

10 **Synthesis of fluorescein-Cys-Gly-Pro-Leu-Gly-Leu-Leu-Ala-Arg-OH linker
conjugate**

Step 1

The peptide component was synthesised on an ABI 433A automatic peptide
15 synthesiser starting with Fmoc-Arg(Pmc)-wang resin on a 0.1 mmol scale using 1
mmol amino acid cartridges. The amino acids were pre-activated using HBTU before
coupling. An aliquot of the peptide resin was then transferred to a clean round bottom
flask an N-methyl morpholine (1 mmol) in DMF (5 ml) added followed by chloroacetyl
chloride (1 mmol). The mixture was gently shaken until Kaiser test negative. The
20 resin was extensively washed with DMF.

Step 2

5 (6)-carboxyfluorescein (188 mg, 0.5 mmol) and dicyclohexylcarbodiimide (113 mg,
0.55 mmol) are dissolved in DMF (20 ml). The mixture is stirred for 2 hours and
25 cooled to 0°C. A solution of hexamethylenediamide (116 mg, 1 mmol) and DMAP (30
mg) in DMF is added and the mixture is stirred at ambient temperature for 72 hours.
The solution is evaporated and the conjugate between carboxyfluorescein and
30 hexamethylene-amine is isolated as monoamide by chromatography (silica,
chloroform and methanol).

Step 3

The resin from step 1 is suspended in DMF (5 ml) and amide-amine conjugate from
step 2 (0.5 mmol) pre-dissolved in DMF (5ml) containing triethylamine (0.5 mmol) is
added. The mixture is heated to 50°C for 16 hours then excess reagents filtered off,
35 following extensive washing with DMF, DCM and diethyl ether then air drying. The
product is treated with TFA containing TIS (5%), H₂O (5%), and phenol (2.5%) for 2
hours.

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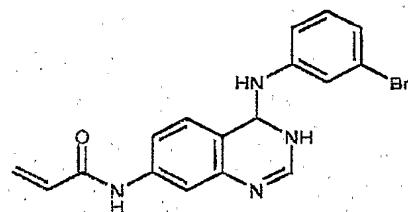
Excess TFA is removed *in vacuo* and the peptide is precipitated by the addition of diethyl ether. The crude peptide conjugate is purified by preparative HPLC C C-18, acetonitril, TFA, water).

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Example 2. Contrast agent for mapping of EGFR/erB-2 tyrosine kinase.

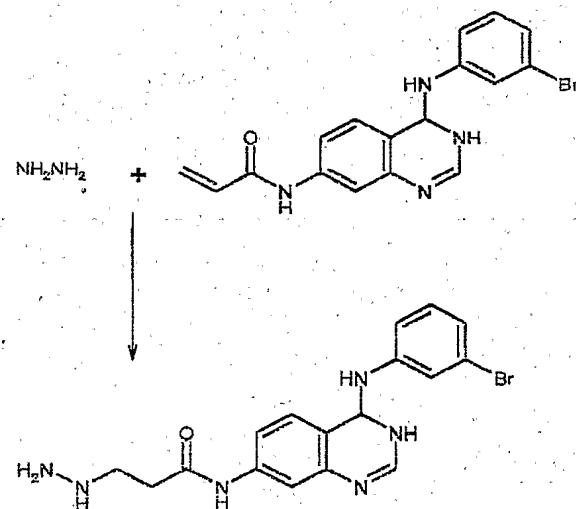
Step 1. N-[4-((3-bromophenyl)amino)quinazolin-7-yl] acrylamide is prepared according to J. B. Smaill *et al* in J. Med. Chem. (1999) **42** 1803-1815.

10



Step 2. N-[4-((3-bromophenyl)amino)quinazolin-7-yl] acrylamide from step 1 (1 mmol) and ethylenediamine (10 mmol) are dissolved in DMF (25 ml). The mixture is stirred at 50 °C for 12 hours. The solvent is evaporated off and the conjugate compound is isolated by flash chromatography (silica, hexane, chloroform, methanol).

15



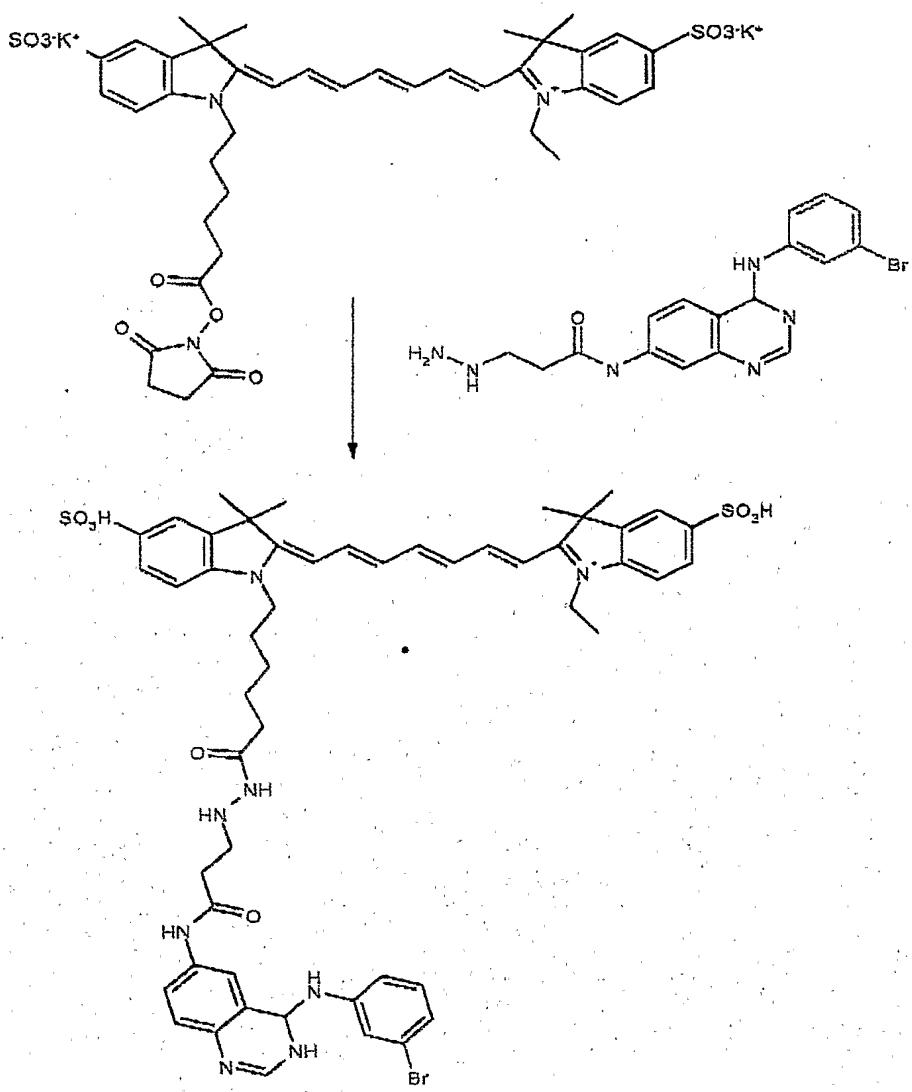
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Step 3. Cy7-NHS ester (0.5 mmol), the conjugate compound from step 2 (0.5 mmol) and N-methylmorpholine (70 mg) are dissolved in DMF (30 ml). The mixture is stirred

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at 40 °C for 3 days. The Cy7 amide conjugate is isolated by flash chromatography (silica, hexane, ethyl acetate, methanol).



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Example 3. Contrast agent for binding to p53 oncoprotein

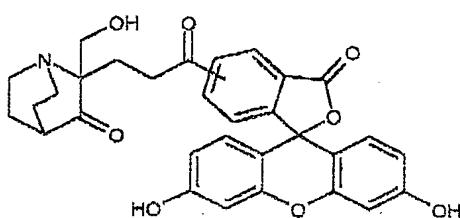
Step 1. Synthesis of 2,2-bis(hydroxymethyl)-1-aza-bicyclo[2.2.2.]octan-3-one.

3-quinuclidinone hydrochloride (Aldrich Q 190-5) (1 mmol) is dissolved in methanol-

water (1:1, 30 ml). An aqueous solution of formaldehyde (37 %, 2.5 mmol) and sodium hydroxide (1.5 mmol) are added. The mixture is stirred for 12 hours at 50 °C. The solvents are evaporated and the title compound isolated as free base using flash chromatography (silica, ethylacetate/chloroform, hexane).

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Step 2.

- 5 5(6)-carboxyfluorescein (0.1 mmol) and dicyclohexyl carbodiimide (0.11 mmol) are dissolved in DMF. The mixture is stirred for 3 hours and cooled to 0 °C. A solution of 2,2-bis(hydroxymethyl)-1-azabicyclo[2.2.2] octane-3-one (0.5 mmol) and DMAP (10 mg) in DMF is added and the mixture is stirred at ambient temperature for 72 hours. The solution is evaporated and the contrast agent is isolate by flash chromatography (silica, ethyl acetate/hexane).
- 10

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Claims:

1. An optical imaging contrast agent with affinity for an abnormally expressed biological target associated with oesophageal cancer or Barrett's oesophagus.

5

2. A contrast agent as claimed in claim 1 with molecular weight below 10000 Daltons.

3. A contrast agent as claimed in claim 1 or 2 of formula I

10

V-L-R, (I)

wherein V is one or more vector moieties having affinity for an abnormally expressed target in oesophageal cancer and Barrett's oesophagus, L is a linker moiety or a bond and R is one or more reporter moieties detectable in optical imaging.

15

4. A contrast agent as claimed in any of claims 1 to 3 comprising a contrast agent substrate, wherein the target is an abnormally expressed enzyme, such that the contrast agent changes pharmacodynamic properties and/or pharmacokinetic properties upon a chemical modification from a contrast agent substrate to a contrast agent product upon a specific enzymatic transformation.

20

5. A contrast agent as claimed in any of claims 1 to 4 having affinity for any of the targets selected from E-cadherin, CD44, squamous cell carcinoma antigen, D, COX-2, matrix metalloproteinases, bombesin receptor, endothelin receptor, P62/c-myc (HGF receptor), p53 and EGFR/erB-2.

25

6. A contrast agent as claimed in any of claims 3 to 5 wherein V is selected from peptides, peptoid moieties, oligonucleotides, oligosaccharides, fat-related compounds and traditional organic drug-like small molecules.

30

7. A contrast agent as claimed in any of claims 3-6 wherein R is a dye that interacts with light in the wavelength region from the ultraviolet to the near-infrared part of the electromagnetic spectrum.

35

8. A pharmaceutical composition for optical imaging for diagnosis of oesophageal cancer and Barrett's oesophagus, for follow up of progress of oesophageal cancer and Barrett's oesophagus development or for follow up of treatment of oesophageal cancer and Barrett's oesophagus, comprising a contrast agent as defined in any of

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claims 1 to 7 together with at least one pharmaceutically acceptable carrier or excipient.

9. Use of a contrast agent as claimed in any of claims 1 to 7 for the manufacture of a

5 diagnostic agent for use in a method of optical imaging of oesophageal cancer and Barrett's oesophagus involving administration of said diagnostic agent to an animate subject and generation of an image of at least part of said subject.

10. A method of generating an optical image of an animate subject involving

10 administering a contrast agent to said subject and generating an optical image of at least a part of said subject to which said contrast agent has distributed, characterized in that a contrast agent as defined in any of claims 1 to 7 is used.

11. Method as claimed in claim 10 for diagnosis of oesophageal cancer and Barrett's

15 oesophagus, for follow up of the progress of oesophageal cancer and Barrett's oesophagus development or follow up of treatment of oesophageal cancer and Barrett's oesophagus using a contrast agent as defined in any of claims 1 to 7.

12. Use of a contrast agent as defined in any of claims 1 to 7 for optical imaging of

20 oesophageal cancer and Barrett's oesophagus.



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Abstract

The invention provides contrast agents for optical imaging of oesophageal cancer and Barrett's oesophagus in patients. The contrast agents may be used in diagnosis 5 of oesophageal cancer and Barrett's oesophagus, for follow up of progress in disease development, and for follow up of treatment of oesophageal cancer and Barrett's oesophagus. Further, the invention provides methods for optical imaging of oesophageal cancer and Barrett's oesophagus in patients.

10

